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Full-Length Genome Characterization of a Novel Simian Immunodeficiency Virus Lineage (SIVolc) from Olive Colobus (*Procolobus verus*) and New SIVwrc*Pbb* Strains from Western Red Colobus (*Piliocolobus badius badius*) from the Taï Forest in Ivory Coast▽

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Simian immunodeficiency viruses (SIVs) are found in an extensive number of African primates and humans continue to be exposed to these viruses by hunting and handling of primate bushmeat. Full-length genome sequences were obtained from SIVs derived from two *Colobinae* species inhabiting the Taï forest, Ivory Coast, each belonging to a different genus: SIVwrc from western red colobus (*Piliocolobus badius badius*) (SIVwrc*Pbb*-98CI04 and SIVwrc*Pbb*-97CI14) and SIVolc (SIVolc-97CI12) from olive colobus (*Procolobus verus*). Phylogenetic analysis showed that western red colobus are the natural hosts of SIVwrc, and SIVolc is also a distinct species-specific lineage, although distantly related to the SIVwrc lineage across the entire length of its genome. Overall, both SIVwrc and SIVolc, are also distantly related to the SIVlho/sun lineage across the whole genome. Similar to the group of SIVs (SIVsyk, SIVdeb, SIVden, SIVgsn, SIVmus, and SIVmon) infecting members of the *Cercopithecus* genus, SIVs derived from western red and olive colobus, L'Hoest and suntailed monkeys, and SIVmnd-1 from mandrills form a second group of viruses that cluster consistently together in phylogenetic trees. Interestingly, the divergent SIVcol lineage, from mantled guerezas (*Colobus guereza*) in Cameroon, is also closely related to SIVwrc, SIVolc, and the SIVlho/sun lineage in the 5′ part of Pol. Overall, these results suggest an ancestral link between these different lentiviruses and highlight once more the complexity of the natural history and evolution of primate lentiviruses.

Simian immunodeficiency viruses (SIVs) are primate lentiviruses that infect an extensive number of wild African primate species. To date, serological and/or molecular evidence for SIV infections have been reported in at least 40 African nonhuman primate species (4–6, 8–12, 15, 16, 19, 22, 23, 29, 31, 33, 37–39, 50, 56, 58, 59). It is now well established that SIVs from chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla gorilla*) in West central Africa and from sooty mangabeys (*Cercocebus atys*) in West Africa are the progenitors of human immunodeficiency virus type 1 (HIV-1) and HIV-2, respectively, the etiologic agents for AIDS (18, 24, 26, 39, 58). These viruses have crossed the species barriers on multiple occasions and generated different groups of HIV-1 (M, N, and O) and HIV-2 (A to H) (21).

Although SIVs are called immunodeficiency viruses by analogy to HIV, they do not induce, with a few exceptions only (30, 36, 55), AIDS-like disease in their natural hosts. This suggests that they have been associated to, and evolved with, their hosts over an extended period of time. Each primate species is generally infected with a species-specific virus, i.e., multiple strains from the same host species form a monophyletic clade. This was used to establish the SIV nomenclature that names the various SIVs by adding a three letters code of their common name indicating the primate species of origin (e.g., SIVcpz from chimpanzee, SIVsmm from sooty mangabey). When different subspecies of the same species are infected, the name of the subspecies is added to the virus designation, e.g., SIVcpz*Ptt* and SIVcpz*Pts* to differentiate between the two chimpanzee subspecies, *P. troglodytes troglodytes* and *P. troglodytes schweinfurthii*, respectively. In some cases, closely related monkey species harbor also closely related SIVs, suggesting that some of these viruses may have coevolved with their hosts for an extended period of time, e.g., L'Hoest and suntailed monkeys from the *lhoesti* superspecies, and the four species of African green monkeys (genus *Chlorocebus*) (2, 4, 7, 22, 57, 61). However, there are also numerous examples of cross-species transmission and recombination, e.g., SIVmnd-2 from mandrills, SIVdrl from drill, SIVagm.sab from sabaeus or even SIVcpz from chimpanzees (3, 8, 25, 43, 46). Interestingly, a single primate species can also be infected by two different SIVs. For example, mandrills from central and southern Gabon are infected with SIVmnd-1, whereas those living in northern Gabon

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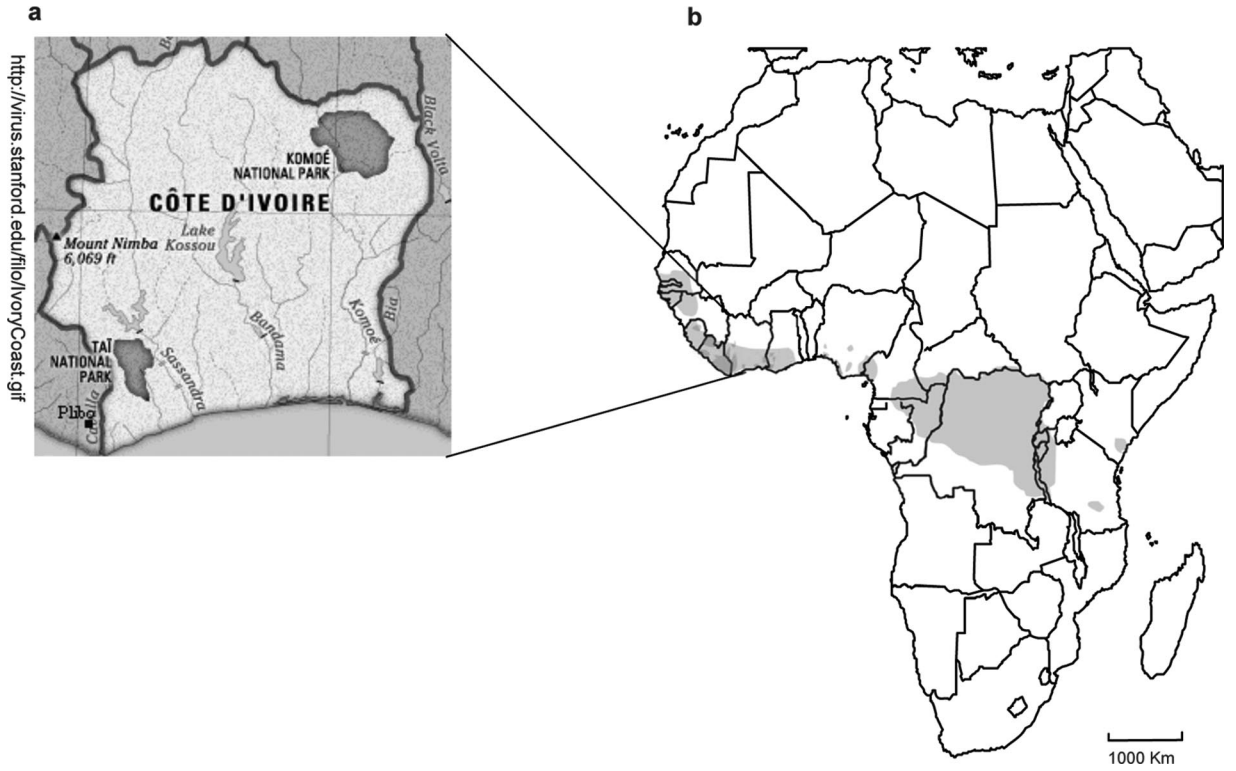

FIG. 1. Location of the Taï National Park in Ivory Coast where the samples were collected (a) and ranges occupied by the different *Piliocolobus* species and subspecies (gray) and *Procolobus* species (dark gray) in Africa (b).

and in south Cameroon are infected with SIVmnd-2 (46, 49). Moreover, it was also recently shown that monkeys living on a small geographic area can be infected by two cocirculating SIV variants, e.g., SIVmus-1 and SIVmus-2 in mustached monkeys from Cameroon (1). These observations indicate that both cross-species transmission and coinfection with highly divergent lentiviral strains are possible and that the evolutionary history of primate lentiviruses has been driven by these successive events over an extended period of time.

To date, with the exception of SIVcpz from chimpanzees and the recently discovered SIVgor from west-central gorillas, all nonhuman primate lentiviruses have been isolated from African Old World monkeys (*Cercopithecidae*), which are subdivided into two distinct subfamilies, *Colobinae* and *Cercopithecinae* (20). *Colobinae* are further separated into African and Asian groups and African colobids are represented by three genera: *Colobus*, *Piliocolobus*, and *Procolobus* (20). Their habitats range over the scattered forested parts of Africa, except for the olive colobus (*Procolobus verus*), which is confined to a limited area of the tropical forest relicts in West Africa only. SIVs have been documented in a very limited number of colobids only, however, from at least one species of each of the three African colobid genera, i.e., SIVcol from black and white colobus (*Colobus guereza*) in Cameroon SIVwrc and SIVolc from Western red colobus (*Piliocolobus badius*) and olive colobus (*Procolobus verus*), respectively, from West Africa (9, 10, 31). Only SIVcol and SIVwrc*Pbt*, from the Western red colobus subspecies (*Piliocolobus badius temminckii*) in The Gambia, have been fully characterized. To date, SIVcol represents the most divergent SIV from all known primate lentiviruses. SIVwrc*Pbt* is a species-specific SIV lineage, although distantly related to the SIVlho and SIVsun lineages across its entire genome (10, 31).

The *Piliocolobus badius* species in West Africa is subdivided into three geographically isolated subspecies: *P. badius badius*, *P. badius waldroni* (nearly extinct today) (35), and *P. badius temminckii*. Partial *pol* and *env* sequences of SIVwrc*Pbb* isolated from Western red colobus (*P. badius badius*) and SIVolc from olive colobus in the Taï forest, Ivory Coast, have been previously described (9, 32). Phylogenetic analyses of these fragments, including the recently described SIVwrc*Pbt* (31), suggest that both SIVwrc*Pbb* and SIVwrc*Pbt* from geographically separate subspecies formed a species-specific monophyletic cluster named SIVwrc lineage (31) and that SIVolc potentially represents a new SIV species-specific lineage (9). To document further the evolutionary history and relationships of SIVs from primates from the *Colobinae* family, we describe here new full-length genomes from two SIVwrc*Pbb* and one SIVolc, from the Taï forest in Ivory Coast.

MATERIALS AND METHODS

Primate specimens and serologic testing. Between 1997 and 2000, blood and tissue samples (kidney, spleen, lung, liver, and lymph node) from nonhuman primate carcasses found on forest floor were sampled in the Taï national park by sanitary surveillance patrols or by primatologists working in the Taï National Park following an Ebola virus outbreak among chimpanzees as previously described (9). This park, situated in south western Ivory Coast, is the largest remaining area of primary forest in West Africa (Fig. 1). Whole-blood and tissue samples from two wild red colobus (98CI04 97CI14) and one wild olive colobus

(97CI12) were available for the present study. The western red colobus samples, 98CI04 and 97CI14, were derived from an adult male of 10 kg and a very old female of 8.5 kg, respectively. The latter died subsequent to a fall from a tree. The olive colobus, 97CI12, was a very old adult female (4.1 kg) killed by an eagle. These animals had previously been shown to be SIV-positive by the presence of cross-reacting antibodies with HIV antigens using Inno-Lia HIV confirmation test and by partial *pol* sequencing (9). Samples were first stored in liquid nitrogen and later kept at −70°C. The identification of the monkeys was done in the field and confirmed by analysis of the skulls.

PCR amplification and sequencing of SIVwrc and SIVolc full-length genomes. For all samples, total DNA was extracted from whole blood and lymph nodes by using the Qiampblood and Qiamptissue kit, and RNA was extracted from plasma for sample 98CI04 by using the QIAampViral RNA minikit according to the manufacturer's instructions (Qiagen SA, Courtaboeuf, France). For the three samples (98CI04, 97CI14, and 97CI12) a small *pol* fragment (650 bp) was initially amplified with a set of degenerate consensus primers as previously described (8).

Similarly as for previous reports on full-length characterization of new SIVs (1, 6, 29, 31), complete SIVwrc*Pbb*-98CI04, SIVwrc*Pbb*-97CI14, and SIVolc-97CI12 genomes were obtained by amplification of overlapping PCR fragments and unintegrated circular DNA using combinations of specific SIVwrc and SIVolc primers, as well as degenerate consensus SIV primers. The primers used are shown in Table 1. For sample 98CI04, specific *pol* primers were designed (Pol0498S1 and Pol0498S2) and reverse transcription-PCR, followed by a semi-nested PCR, was performed to amplify a 3,000-bp fragment spanning the end of *pol*, accessory genes, and the beginning of *env* with a combination of specific and degenerate primers: Pol0498S1/SIV-ER1 for the first round and Pol0498S2/SIV-ER1 for the second round. New specific *env* primers were then designed (0498ENVS1 and 0498ENVS2), and a seminested PCR was performed with SIVnef-as as the reverse primer to obtain a PCR fragment (~1,300 bp) spanning the end of *env* and the first half of *nef*. On the basis of the relationship observed between SIVwrc*Pbb*-98CI04 and viruses from the SIVlho lineage in the *env* phylogeny, consensus primers were designed (ENVF2LHO and ENVR2LHO). For sample 97CI14, a similar PCR amplification strategy was applied for the amplification of the 3′ end of *pol* up to the first half part of *nef*. Briefly, we performed a nested and seminested PCR with generic SIVwrc *pol* primers and modified SIV-ER1 primer (SIVenvR) for the first round and ENVF2LHO/ENVR2LHO, followed by WRCenvS2/SIVenvR and PolwrcolF2/WRCenvR1, for the second round. We then performed a seminested PCR with new *env*-specific primers (1497EnvS4 and 1497EnvS5) and generic *nef* antisense primer (SIVnef-as). We defined new SIVwrc generic primers for *env* and carried out PCR amplifications from unintegrated circular DNAs for both samples (98CI04 and 97CI14) using PolwrcolR1/EnvwrcolF1 for the first round, followed by PolwrcolR2/ENVwrcolF2 for the second round. For sample 97CI12, we amplified a 756-bp *pol* PCR fragment using generic SIVwrc *pol* primers as previously described (32) and designed new SIVolc-specific *pol* forward and reverse primers. We then performed nested PCRs spanning the 3′ end of *pol*, the accessory genes, and the 5′ beginning of *env* with specific *pol* primers and modified *env* primers (POLF1-1297/SIVenvR for the first round and POLF2-1297/ENVR1-1297 and ENVLHOF2/ENVLHOR2 for the second rounds). In parallel to these PCRs, we amplified a region spanning the second half of *gag* up to the end of *pol* using generic and specific primers (SPBS/1297-POLR1 for the first round, followed by SIVgagS/1297-POLR4 and 1297gagF1/1297PolR2 for the second rounds). We then designed new *gag*- and *env*-specific primers and amplified unintegrated circular DNA.

Reverse transcription-PCR and PCR amplifications were performed by using Expand reverse transcriptase and the Long Expand PCR kit, respectively (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Each amplification reaction included a manual hot-start, followed by 35 to 40 cycles. Annealing temperatures were set according to the primer melting temperatures, and extension times varied depending on the sizes of the expected fragment and were typically set at 1 min/kb. PCR products were agarose gel purified and directly sequenced by using cycle sequencing and dye terminator methodologies (ABI Prism BigDye terminator cycle sequencing ready reaction kit with AmpliTaq FS DNA polymerase [PE Biosystems, Warrington, England]) on an automated capillary sequencer (ABI 3130*XL*; Applied Biosystems). To reconstitute the full-length genome sequence, overlapping sequences were assembled into contiguous sequences by using SeqMan DNAStar software (Lasergene; DNAStar, Inc., Madison, WI).

Sequence similarity plots. Nucleotide and protein sequences were aligned by using MEGA3 and CLUSTALX 1.8 (28, 52), with minor manual adjustments. Sites that could not be unambiguously aligned were excluded. Proteome sequences were generated by joining deduced Gag, Pol, Env, and Nef amino acid sequences; the carboxy-terminal Gag and Env amino acid sequences that overlapped with Pol and Nef amino acid sequences respectively, were excluded. The predicted protein sequences encoded by SIVwrc*Pbb* and SIVolc were compared to representatives of known HIV/SIV lineages. In order to study whether the newly characterized SIVwrc*Pbb* and SIVolc sequences were recombinant with any of the other SIV lineages, similarity plot analysis was performed with the SIMPLOT package version 2.5 (41) using a sliding window of 200 amino acids (aa) moved in steps of 20 aa.

Phylogenetic analyses. Amino acid sequence regions used for phylogeny reconstruction were defined on the basis of the simplot results and were as follow: Gag (390 aa), Pol1 (279aa), Pol2 (286 aa), Pol3 (355 aa), and Env (560 aa). Phylogenies were inferred by the Bayesian method implemented in MrBayes v3.1 (64) and run for 3, 5, and 6 million generations for Gag, Pol (Pol1, Pol2, and Pol3), and Env proteomes, respectively, with a 10% burn-in. The mixed model in MrBayes indicated that the rtRET model of amino acid change (14) was most appropriate; this model was thus used with gamma distribution rates across sites (63). Parameters were examined with the Tracer program (Evolutionary Biology Group, Oxford University, Oxford, United Kingdom; http://evolve.zoo.ox.ac.uk/software.html).

RNA Secondary structure predictions. The TAR RNA secondary structure was predicted and drawn by using the GenQuest DNAStar package (Lasergene; DNAStar).

Nucleotide sequence accession numbers. The complete sequences have been deposited to the GenBank under the following numbers: SIVwrc*Pbb*-04CI98 (AM713177), SIVwrc*Pbb*-14CI97 (AM745105), and SIVolc-12CI97 (FM165200, FM165201, and FM165202).

RESULTS

Genomic organization and functional motifs of SIVwrc*Pbb* and SIVolc. SIVwrc*Pbb* and SIVolc full-length genomes were compared to other primate lentiviruses and showed the expected reading frames for *gag*, *pol*, *vif*, *vpr*, *tat*, *rev*, *env*, and *nef* and did not encode for a *vpu* or *vpx* analogue. The SIVwrc*Pbb* and SIVolc long terminal repeats (LTRs) contain all of the characteristic features of other primate lentivirus LTRs, including TATA, NF-κB sites, and potential SP-1 regions. The secondary structure prediction of SIVwrc*Pbb* TAR showed an unusual organization with a double stem-loop structure consisting of a three nucleotides (GCC) and a single-nucleotide bulge (U) and 7- and 6-bp stems with a 5-bp identical terminal loop with the sequence 5′-CUGGU-3′. Despite some differences, this TAR element is quite similar to the one previously described for SIVwrc*Pbt* from a western red colobus of the *P. badius temminckii* subspecies in The Gambia (31), which reinforces the common origin of these viruses. In turn, SIVolc has a specific and typical predicted secondary structure of TAR element with two stem-loops consisting of two identical nucleotide bulges (UU) and two 6-bp stems with a 6-bp terminal loop with the sequences 5′-CUGAGU-3′ and 5′-CUGGGU-3′, respectively.

Like all other known primate lentiviruses, SIVwrc*Pbb* and SIVolc contain 18 cysteine residues conserved across the gp120 envelope glycoprotein surface subunit. Interestingly, the additional cysteine residues described in the SIVlho/sun lineage, SIVdrl/mnd-2 and SIVwrc*Pbt*, are also conserved in both SIVwrc*Pbb* and SIVolc. Finally, two different binding sites known to be critical for primate lentivirus budding have been identified in SIV Gag p6 protein sequences: PT/SAP and YPXL (17, 34, 40, 48, 60). With the exception of SIVdeb and SIVden, both motifs (PT/SAP and YPXL) are found in *Cercopithecus* and *Miopithecus* SIV lineages (6, 12, 29) and have been proposed to constitute a specific signature for the *Cercopithecus* SIV lineage (6). Although both motifs are present in SIVwrc*Pbt* (31), the YPXL motif is absent in SIVwrc*Pbb* and

TABLE 1. Primers used to amplify full-length genomes of SIVwrc*Pbb*-98CI04, SIVwrc*Pbb*-97CI14, and SIVolc-97CI12

Fragment[b]	Fragment size (bp)	PCR round	Primer (sequence [5′–3′])[a]	Reference(s)
SIVwrc*Pbb*-98CI04				
A. End of *pol*		First	NDR1 (TRGAYACAGGRGCWGAYGA)	8, 10
			PolOR (ACBACYGCNCCTTCHCCTTTC)	8, 10
	655	Second	Polis4 (CCAGCNCACAAAGGNATAGGAGG)	8, 10
			Uni2 (CCCCTATTCCTCCCCTTCTTTTAAAA)	8, 10
B. 3′*pol* to 5′*env*		First	Pol0498S1 (AAGCCATTTGCTGGTGGTTAG)	
			SIV-ER1 (TTNYKCTGYTGCTGCACTATCCCAG)	
	3,089	Second	Pol0498S2 (TATAATCCTCAGAGCCAAGGAG)	
			SIV-ER1	
C. End of *env* to 5′*nef*		First	0498ENVS1 (TTTCCACAGGGTACTACAAAGAGG)	
			SIVnef-as (CAGTCCHCCCTTTTCTTT)	
	1,300	Second	0498ENVS2 (TGGAAAGAGCAGCAGGAGATCAGG)	
			SIV-nef-as	
D. 3′*env* to *pol* (circular DNA)		First	PolwrcolR1 (GCCATWGCYAATGCTGTTC)	32
			EnvwrcolF1 (TGGCAGTGGGACAAAAATATAAAC)	32
	~6,000	Second	PolwrcolR2 (GTTCWATTCCTAACCACCAGCADA)	32
			EnvwrcolF2 (TGATAGGGMTGGCTCCTGGTGATG-3′)	32
SIVwrc*Pbb*-97CI14				
A. End of *pol*		First	NDR1-PolOR	8, 10
	655	Second	Polis4-Uni2	8, 10
B. 3′*pol* to 5′*env*		First	PolwrcolF2 (AGAGACAGTAAGGAAGGGAAAGCAGG)	32
			SIV-envR (YTBYTGCTGCTGCAMTATCCC)	
B1. 5′*env*	572	Second	SIVLHOF2 (AATCAGATAGTNYAGCAAGCATGG)	
			SIVLHOR2 (CCATTAAAKCCAAAGAAGCTACT)	
B2. 3′*env*	1,418	Second	WRCenvS2 (CACCCTATTGTGTAAAAATGMAMTGTAC)	
			SIV-envR	
B3. 3′*pol* to 5′*env*	2,408	Second	PolwrcolF2	32
			WRCenvR1 (GATTTTTACACAWGGCTTTAATAAGG)	
C. 3′*env* to 5′*nef*		First	1497EnvS4 (AAAATGATAGGGATGGCACCAGG)	
			SIVnef-as	
	1,177	Second	1497EnvS5 (GTACCCCCTGAACATCGCAGAG)	
			SIVnef-as	
D. 3′*env* to *pol* (circular DNA)		First	PolwrcolR1	32
			EnvwrcolF1	32
D1. 3′*env* to *pol* (circular DNA)	~6,000	Second	PolwrcolR2	32
			EnvwrcolF2	32
D2. 3′*env* to LTR	2,031	Second	EnvwrcolF2	32
			SPBSrev (CAAGTCCCTGTTCGGGCGCC)	
D3. *pol*	2,319	Second	NDRI	8, 10
			PolwrcolR2	32
SIVolc-97CI12				
A. 3′*pol*		First	PolwrcolF1	32
			PolwrcolR1	32
	754	Second	PolwrcolF2	32
			PolwrcolR2	32
B. 3′*pol* to 5′*env*		First	1297PolF1 (CCAGCACAAGAGAGCCACAATAAGTATCAT)	
			SIV-envR	
B1. 3′*pol* to 5′*env*	2,083	Second	1297PolF2 (AGTGGCAAAAAGGATAGTAGATGAATGTGA)	
			1297envR1 (GCTTTTCTGTTTCTGGCTTTACTGT)	
B2. 5′env	557	Second	SIVLHOF2	
			SIVLHOR2	
C. 3′LTR to *pol*		First	SPBS (GGCGCCCGAACAGGGACTTG)	
			1297PolR1 (TTGATCTACTTCTTGATTGCCCCCT)	
C1. 3′*gag* to 5′*pol*	2,154	Second	SIV-Gags (GCHTGYCAAGGAGTGGGAGGNCC)	
			1297PolR4 (TAATACTGTAATCTGTTCCCCTTCTTTT)	
C2. End of *gag* to *pol*	1,312	Second	1297gagF1 (TGTGACAGATTGGAAGAAGAAGGTA)	
			1297PolR2 (CTGCTTTCTGATTACTACTCTCCTG)	
D. 3′*env* to 5′*gag* (circular DNA)		First	1297envF1 (AGACCACTACAATAGGGTTAGGAGT)	
			1297gagR1 (GCTCTGTTGTTGTTGTCCTCCCACC)	
5′LTR to 3′*gag*	1,297	Second	SPBS (GGCGCCCGAACAGGGACTTG)	
			1297gagR2 (TGAACAATTATTTTTAGTATGCCCA)	
E. 3′*env* to 5′*gag*		First	1297envF1	
			1297gagR3 (CCTGTCTTCCAATGCTTTTACCCAA)	
	~3,000	Second	1297envF2 (ACAGTAAAGCCAGAAACAGAAAAGC)	
			1297gagR4 (TTCTTTTACAGCCTTCCTTAGTTCC)	

[a] Y = C or T; W = A or T; R = A or G; H = A or C or T; B = C or G or T; M = A or C; S = G or C; K = G or T; V = G or A or C; D = G or A or T; N = A or G or C or T; I = inosine.

[b] The letters "A," "B," "C," etc., correspond to the different fragments amplified by the different nested PCR systems for each new SIV.

is replaced by a WPXL motif. In contrast, an YPXL motif is present in the SIVolc, thereby increasing the number of non-*Cercopithecus* SIVs with both PT/SAP and YPXL motifs.

Sequence similarity analyses. In order to compare the new full-length SIVwrc*Pbb* and SIVolc sequences to previously characterized SIV strains, we performed similarity plot analyses on concatenated proteomes, including Gag, Pol, Env, and Nef (Fig. 2 and 3). The accessory genes region, including Vif, was omitted from the alignment due to high sequence heterogeneity and low signal information. Figures 2a and 3a show

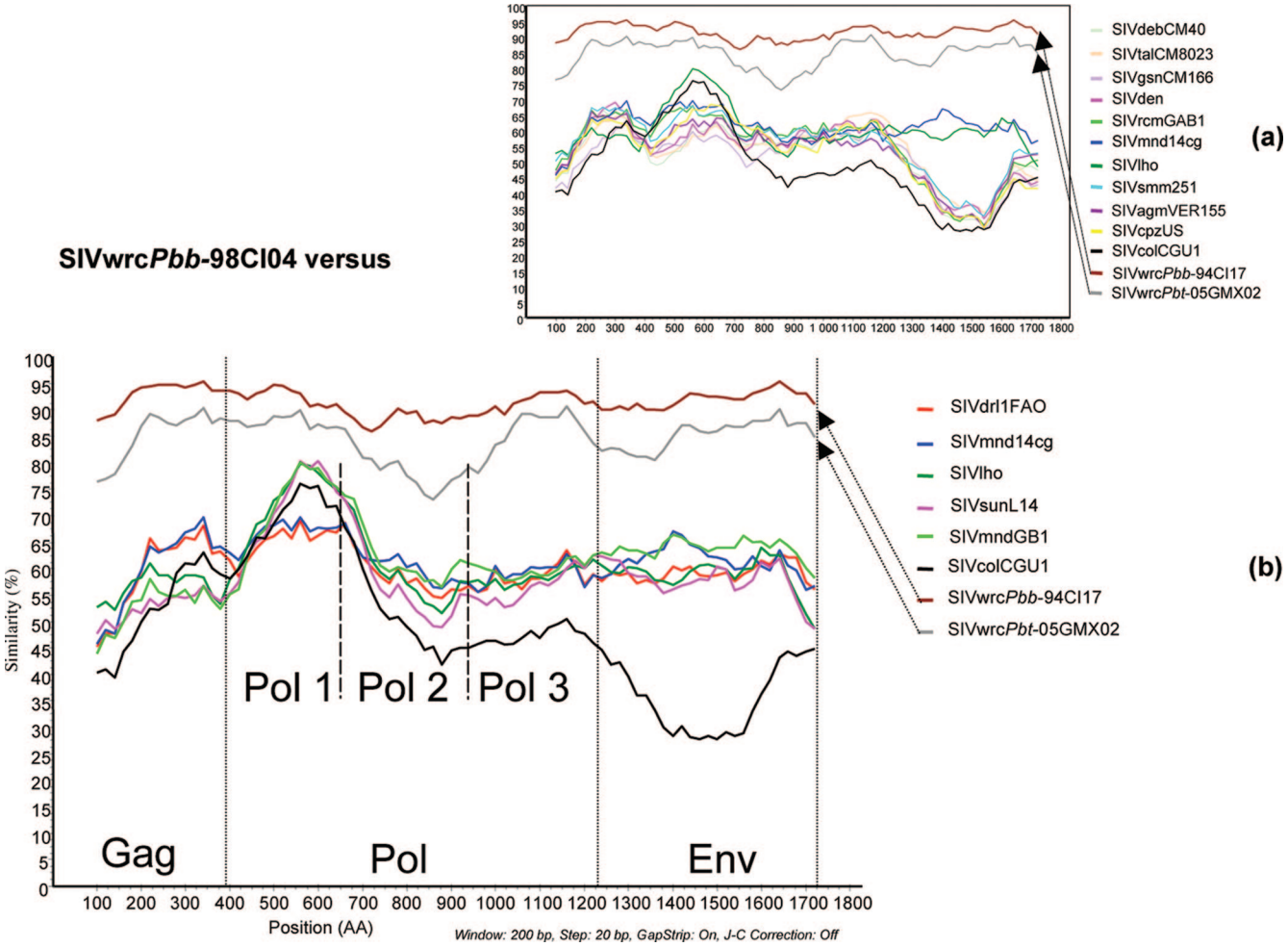

FIG. 2. Similarity plots of concatened Gag, Pol, Env, and Nef protein sequences showing similarities between SIVwrc*Pbb* versus other SIVs representative for the different SIV lineages, obtained with a sliding window of 200 aa moved in steps of 20 aa. SIV lineages with a high interest for our study, namely, SIVwrc*Pbt*, SIVlho, SIVsun, SIVdrl, SIVmnd-2, and SIVcol, are shown in the large-scale window (b), whereas the comparison with all of the representative SIV strains known is represented in the smallest window (a). The vertical axis shows the percentage of similarities, and the horizontal axis shows the amino acid positions.

that, depending on the parts of the genome studied, the new SIVwrc*Pbb* and SIVolc lineages are most closely related to SIVwrc*Pbt*, SIVlho, SIVsun, SIVmnd-2, SIVdrl, and SIVcol. For clarity, representatives of the SIV lineages most relevant to our study are shown separately in Fig. 2b and 3b.

Figure 2b depicts in more detail similarities between SIVwrc*Pbb*-98CI04 and SIVwrc*Pbb*-97CI14, as well as with the other representative relevant SIV lineages. The two SIVwrc*Pbb* strains were quite similar to one another and shared 81 to 95% amino acid identity depending on the gene analyzed, as shown in Table 2. Across their entire genomes, SIVwrc*Pbb* were also closely linked to the recently characterized SIVwrc*Pbt* from the geographically separated *temminckii* subspecies in the Gambia (31). As described for SIVwrc*Pbt*, SIVwrc*Pbb* strains were thus also more closely related to the SIVlho/sun lineage than to any other SIV, particularly in two parts of their proteomes corresponding to the 5′ part of Pol and to the entire Env.

Figure 3b shows similarities between SIVolc from olive colobus and the other representative SIV strains. SIVolc is related to SIVwrc across almost the entire genome, and SIVolc is thus also related to the SIVlho lineage in the regions corresponding to the 5′ part of Pol and over the entire Env. In addition, the similarity plots show also that SIVcol is closer to the SIVwrc, SIVolc, and SIVlho lineages than to SIVs from other monkey species in the N-terminal part of Pol. In the Pol protein, three different patterns are observed: in the N-terminal part as well as in the C-terminal part of the Pol region, SIVolc seemed to be more closely related to SIVwrc strains, whereas in the middle part of the Pol region the SIV relationships were unclear. Overall, these results suggest a complex evolutionary history between ancestral SIVs in the *Colobinae* subfamily and strains from the SIVlho/sun lineage, possibly driven by cross-species transmission and recombination events over an extended period of time.

Phylogenetic analyses of full-length SIVwrc*Pbb* and SIVolc genomes. The different phylogenetic trees show that SIVwrc*Pbb* and SIVolc are each distinct species-specific SIV lineages but distantly related across their entire genomes. SIVwrc*Pbb* forms a monophyletic species-specific lineage with the recently de-

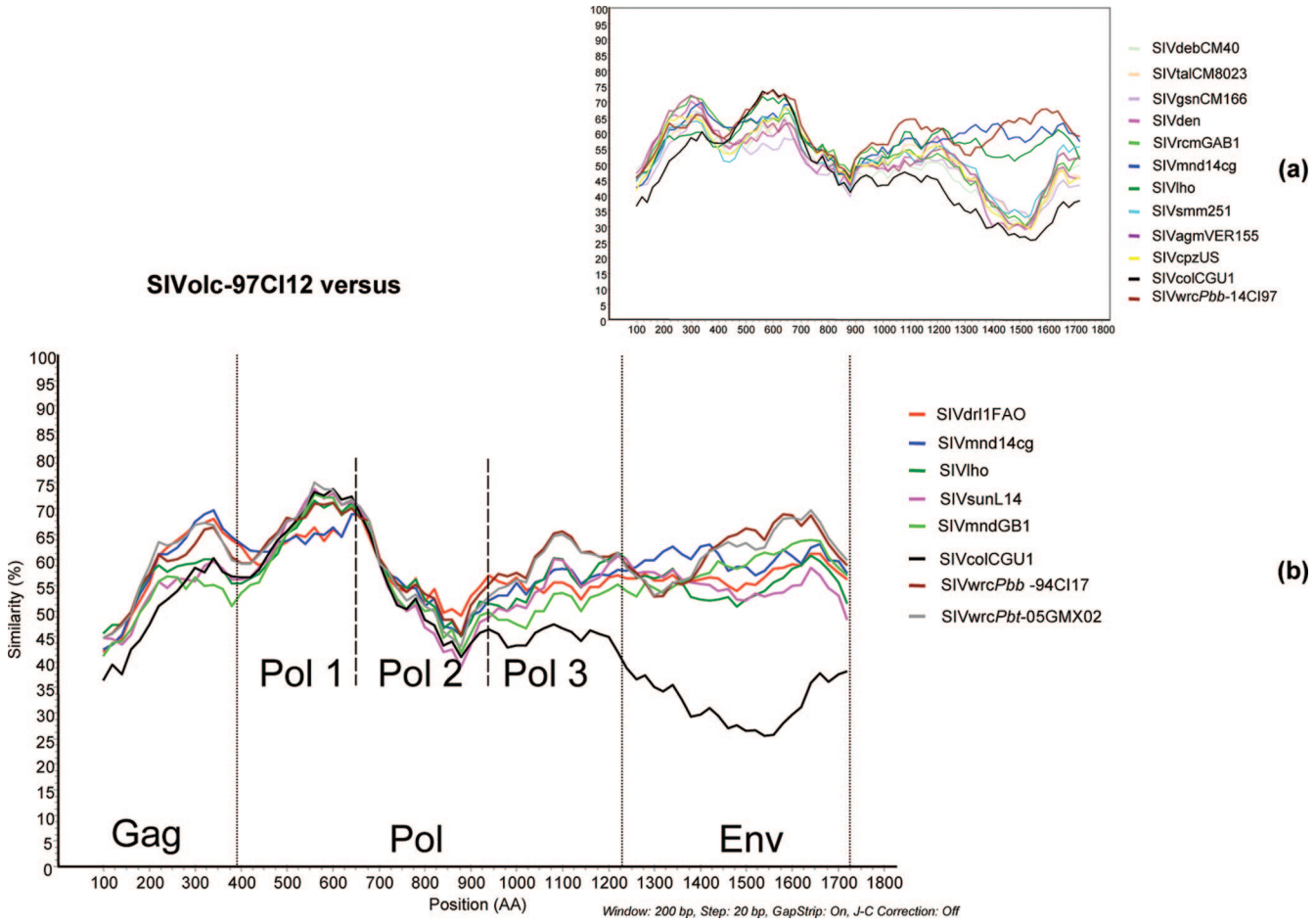

FIG. 3. Similarity plots of concatened Gag, Pol, Env, and Nef protein sequences showing similarities between SIVolc versus other SIVs representative for the different SIV lineages, obtained with a sliding window of 200 aa moved in steps of 20 aa. SIV lineages with a high interest for our study, namely, SIVwrc, SIVlho, SIVsun, SIVdrl, SIVmnd-2, and SIVcol, are shown in the large-scale window (b), whereas the comparison with all of the representative SIV strains known is presented in the smallest window (a). The vertical axis shows the percentage of similarities, and the horizontal axis shows the amino acid positions.

scribed SIVwrc*Pbt* strain (31) in the phylogenies obtained with each of the three major proteins (Fig. 4), as well as in the accessory proteins (data not shown). For more detailed phylogenetic analysis, the Pol protein has been subdivided into three fragments, according to the observations of the similarity plot analysis of SIVolc versus the other SIVs.

For each protein analyzed, both SIVwrc and SIVolc lineages clustered together, although distantly related to each other, but also distantly related to the SIVlho lineage that includes SIVlho and SIVsun from L'Hoest and sun-tailed monkeys and SIVmnd-1 from mandrills. In Gag (Fig. 4a), the highest level of divergence is observed between the above-mentioned SIV lineages. Interestingly, in all phylogenies except in Pol2, SIVolc conserves a basal position compared to SIVwrc. In the Gag and Pol1 trees (Fig. 4a and b), SIVcol from *Colobus guereza* clustered also with SIVwrc, SIVolc, and SIVlho lineages. Nevertheless, in the *gag* gene, this observation must be viewed with caution due to the high degree of divergence characterized by the long branches within this clade, as well as a low posterior probability value (≥91%). In the Pol1 tree, the relationships between SIVwrc, SIVolc, SIVlho, and SIVcol is supported by high posterior probability values and suggests a clear ancestral link between these different SIV lineages in this part of the genome. Genetic distance analysis strengthens this observation with 68 to 71% amino acid identities between SIVwrc, SIVlho, SIVolc, and SIVcol lineages (Table 2).

In the Env protein, SIVwrc and SIVolc lineages form a highly supported cluster with the SIVlho lineage, as well as with SIVmnd-2 and SIVdrl, which are known to cluster with SIVmnd-1 and SIVlho/sun in Env. Interestingly, in Env, SIVwrc and SIVolc appear to be more closely related to SIVmnd-1/mnd-2/drl than to SIVlho/sun. Amino acid identities (56 to 61% versus 51 to 54%) further illustrate this relationship (Table 2).

In addition to the group of SIVs (SIVsyk, SIVdeb, SIVden, SIVgsn, SIVmus, and SIVmon) that infects members of the *Cercopithecus* genus, we observed that SIVs derived from western red and olive colobus, mandrills, and L'Hoest and sun-tailed monkeys, form a second group of viruses which cluster consistently together in phylogenetic trees. We also observed fluctuating relationships for other lineages across the different tree topologies. For example, the SIVcpz lineage that is de-

TABLE 2. Percent amino acid identity between SIVwrc*Pbb* (04CI98 and 14CI97), SIVolc (12CI97), and SIV strains representative of other SIV lineages in the three major fragments, Gag, Pol, and Env

SIV strain	% Amino acid identity[a]														
	Gag			Pol1			Pol2			Pol3			Env		
	SIVolc 97CI12	SIVwrc*Pbb* 94CI17	SIVwrc*Pbb* 98CI04	SIVolc 97CI12	SIVwrc*Pbb* 94CI17	SIVwrc*Pbb* 98CI04	SIVolc 97CI12	SIVwrc*Pbb* 84CI17	SIVwrc*Pbb* 98CI04	SIVolc 97CI12	SIVwrc*Pbb* 94CI17	SIVwrc*Pbb* 98CI04	SIVolc 97CI12	SIVwrc*Pbb* 94CI17	SIVwrc*Pbb* 98CI04
SIVdebCM40	55	54	54	58	55	54	49	54	54	48	56	57	39	37	38
SIVtalCM8023	54	54	54	56	57	55	53	54	56	53	60	62	39	39	40
SIVlsnCM166	51	49	49	56	56	56	48	52	52	51	62	60	39	35	37
SIVmonCML1	52	52	52	56	56	56	49	49	49	49	58	58	39	36	37
SIVmusCM1085	52	51	52	57	58	56	46	56	54	50	60	59	38	36	37
SIVden	57	56	58	57	59	56	49	52	54	51	59	60	37	37	38
SIVrcmNlm	54	52	53	63	65	65	55	59	60	51	60	60	39	38	38
SIVsyk173	51	51	51	57	59	58	44	50	49	54	57	57	40	39	40
SIVsmm251	51	55	56	61	64	64	48	56	56	52	59	58	42	42	42
SIVagmVER155	57	54	55	58	59	58	49	56	56	52	58	57	40	41	41
SIVclzUS	53	52	53	60	61	60	52	60	57	52	59	59	38	36	36
SIVdrl1FAO	52	53	54	64	66	65	54	57	57	53	58	58	54	***56***	***57***
SIVmnd14cq	52	54	55	64	66	65	53	60	60	55	59	58	57	***57***	***59***
SIVlho	51	55	55	65	**70**	**70**	52	60	58	56	58	58	51	***54***	***53***
SIVsunL14	49	52	50	67	**68**	**69**	48	57	56	55	58	57	51	***54***	***53***
SIVmndGB1	47	49	49	66	**71**	**69**	50	58	60	51	60	60	56	***59***	***61***
SIVolc97CI12	100	53	54	100	**67**	**68**	100	54	54	100	59	58	100	***58***	***58***
SIVwrc*Pbb*-94CI17	53	*100*	*91*	67	*100*	*91*	54	*100*	*87*	59	*100*	*92*	58	*100*	*91*
SIVwrc*Pbb*-98CI04	54	*91*	*100*	68	*91*	*100*	54	*87*	*100*	58	*92*	*100*	58	*91*	*100*
SIVwrc*Pbt*-05GMX02	54	*82*	*82*	68	*84*	*86*	51	*75*	*77*	58	*86*	*86*	59	*83*	*84*
SIVcolCGU1	45	48	49	67	**68**	**67**	50	49	49	44	46	48	31	33	34

[a] Italic letters show the percentages of amino acid identity among the three strains of SIVwrc (SIVwrcPbb-98CIO4, SIVwrcPbb-97CI14, and SIVwrcPbt-05GMX02) for the three major gene products, Gag, Pol, (Pol1, Pol2, and Pol3), and Env. For the first part of Pol (Pol1), the percentages of amino acid identity among SIVwrcPbb, the SIVIho lineage, SIVolc, and SIVcol are shown in bold, and for Env, the percentages of amino acid identity among SIVwrcPbb, the SIVlho lineage, SIVolc, SIVdrl/mnd-2 are shown in bold italics.

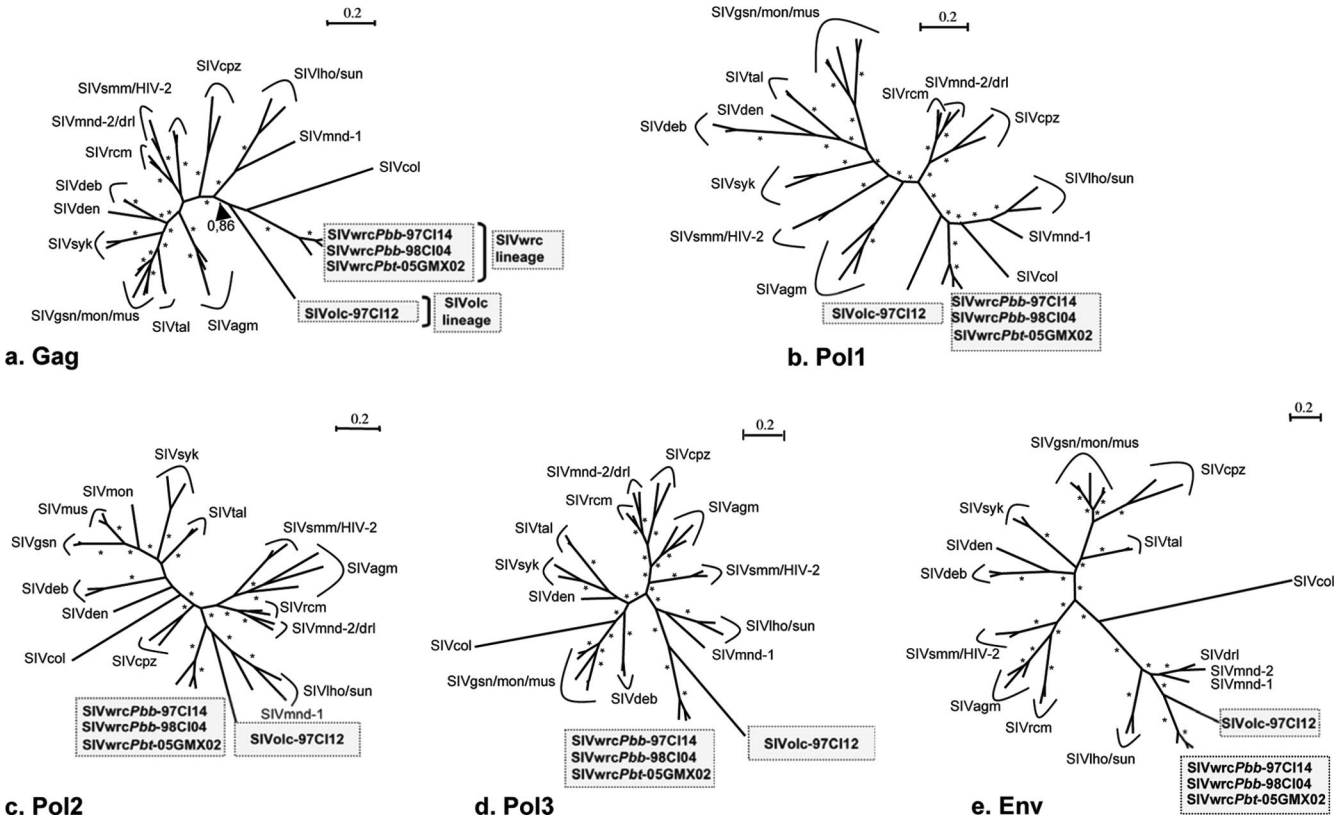

FIG. 4. Phylogenetic relationships between SIVwrc*Pbb* and SIVolc with other representative SIV lineages in Gag (a), Pol (b, c, and d), and Env (e) genes. Phylogenies were inferred by using the Bayesian method. Stars at node represent posterior probabilities. Only those at ≥91% are shown. Scale bars indicate substitutions per site.

scribed as a recombinant strain between both SIVrcm and SIVgsn ancestor lineages forms a distinct monophyletic group in Gag and Pol2 tree, thus suggesting a more complex natural history and evolution than previously described (3). Overall, these results highlight the complexity of disentangling the phylogenic relationships within the primate lentiviruses as more genomic data become available.

DISCUSSION

In this study we describe the full-length genome sequences for SIVs derived from two *Colobinae* species, each belonging to a different genus, SIVwrc from western red colobus (*Piliocolobus badius badius*) and SIVolc from olive colobus (*Procolobus verus*) inhabiting the Taï forest in the south-eastern part of Ivory Coast. We confirmed that geographically isolated subspecies of the western red colobus, in The Gambia and Ivory Coast are infected with closely related species-specific SIVs, and that Western red colobus are thus the natural hosts of SIVwrc (31). We also showed that SIVolc is a distinct species-specific lineage, but more closely related to the SIVwrc lineage than to any other SIV across almost the entire length of its genome. Overall, SIVs derived from western red and olive colobus, mandrills, L'Hoest and suntailed monkeys, form a group of viruses that cluster consistently together in phylogenetic trees.

The common evolutionary history of SIVwrc*Pbb* and SIVwrc*Pbt* is not surprising because animals of the two *Piliocolobus* subspecies may have shared gene flow until recently, and their ranges, which are poorly documented, could still overlap (53). The genetic diversity between SIVolc and SIVwrc is significantly higher than among SIVs from the different subspecies of western red colobus and is thus most likely the result of an ancient cross-species transmission or an infection by a common ancestor. It will thus be important to characterize additional SIVolc strains from wild olive colobus in other geographic areas in order to determine to what extent this species is infected with SIV and also to identify whether SIVolc is the result of an ancestral cross-species transmission between red colobus and olive colobus from the Taï forest in Ivory Coast, the only region where their habitats overlap. It will also be interesting to study more in detail western red and olive colobus in the Taï forest, where they live in polyspecific primate associations, to examine to what extent cross-species transmissions still occur.

Overall, SIVwrc and SIVolc are most closely related to the SIVlho/sun lineage across the whole genome. Interestingly, SIVcol, which represents a divergent SIV lineage, is also closely related to SIVwrc, SIVolc, and the SIVlho lineage in the 5′ part of Pol and to a lower extent in Gag. The relationships between these different SIV lineages are somewhat surprising because of the geographical separation of their hosts. The characterization of these new SIVwrc and SIVolc lineages raises thus more questions than answers

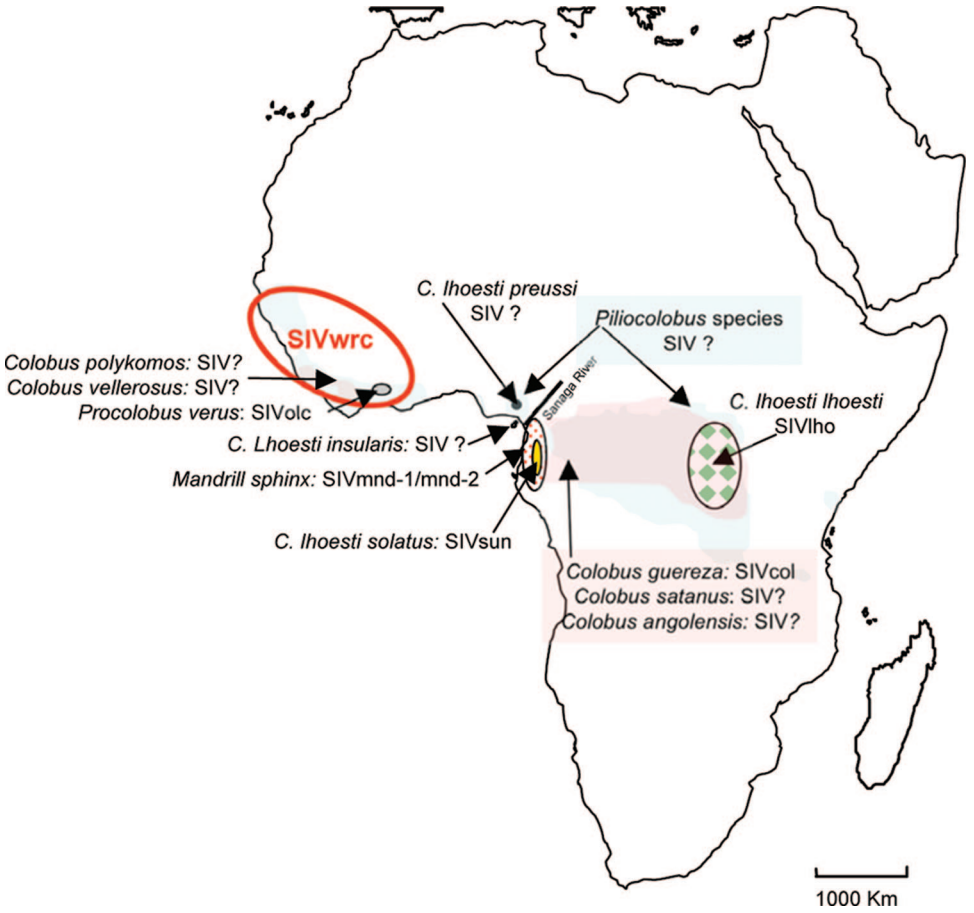

FIG. 5. Ranges occupied by the different species and subspecies of *Piliocolobus* (in blue), including those overlapping with the *C. lhoesti* superspecies (*C. lhoesti*, *C. solatus*, *C. preussi*, and *C. insularis*) and the colobus monkeys (in red).

regarding the evolution of SIVs. The three species carrying SIVs from the SIVlho lineage (SIV mnd-1 from *M. sphinx*, SIVlho *from C. lhoesti*, and SIVsun from *C. solatus*) are all confined to Central Africa. The ranges of the different *Piliocolobus* and *Colobus* species and subspecies cover discontinuously the west-African forest blocks extending into central-eastern Africa (Fig. 1 and 5). Although the actual range of certain *Piliocolobus* and *Colobus* species overlaps that of *C. lhoesti* superspecies to the east of the Democratic Republic of Congo and to the southwest of Cameroon, their habitats do not overlap at all with the West African species (Fig. 5). In order to better understand the evolution of SIVs in the colobines, it will be important to characterize additional SIVs in the remaining species of *Colobus* and *Piliocolobus* genera across Africa. Particular attention should be paid to *Piliocolobus* and *Colobus* species whose ranges overlap today with those of the *Cercopithecus* species harboring SIVlho and SIVsun. This will help to determine whether the virus emerged before or after speciation or geographic separation events among colobids and will provide further insight into the importance of biogeographic barriers and cross-species transmission in SIV evolution (Fig. 5). Especially, it will be important to study whether black and white colobus (*Colobus polykomos*) species in the Taï forest are infected and characterize their SIV.

It is now well established that the evolutionary history of primate lentiviruses has been driven by host-virus coevolution, cross-species transmission, and recombination events over an extended period of time. Indeed, the description of new SIVwrc and SIVolc strains renders the evolutionary history of primate lentiviruses even more difficult to disentangle but shows that apparently two major groups of SIV lineages can be observed: one previously described that comprises the SIVs from the majority of the *Cercopithecus* species (6) and one observed in the present study comprising SIVs from western red and olive colobus, SIVs from L'Hoest and suntailed monkeys, and SIVmnd-1 from mandrills. This suggests that there are two SIV lineages in *Cercopithecus*: one for arboreal and one for semiterrestrial species (*C. lhoesti* and *C. solatus*). However, studies on the evolution of the primate hosts show that *C. lhoesti* and *C. solatus* do not cluster with the other species of the *Cercopithecus* genus but form a clade with semiterrestrial species (*Erytrocebus* and *Chlorocebus* spp.), suggesting a taxonomic revision for *C. lhoesti* and *C. solatus* (54, 62). The majority of colobids are arboreal species, and therefore the distinction between an arboreal and a terrestrial SIV lineage cannot be generalized beyond the actual *Cercopithecus* genus. However, geographic isolation and ecological factors such as vegetation type and distribution can shape or elicit new or different behaviors and excep-

tional cases of colobids with semiterrestrial behavior and living in polyspecific associations with semiterrestrial species have been documented, e.g., *P. badius temminckii* in The Gambia (13, 31). These different polyspecific associations could play a role in cross-species transmission and recombination of divergent SIVs and explain the clustering of SIVwrc, SIVolc, SIVlho, SIVsun, and SIVmnd-1.

In addition to the relationship of SIVs from colobids from West Africa and L'Hoest and suntailed monkeys from Central Africa, the relationship between SIVlho/sun and SIVmnd-1 remains also an enigma. Mandrills and monkeys from the L'Hoest superspecies are phylogenetically distant species within the *Cercopithecinae* and inhabit geographically separate regions of Central Africa. Only the range of SIVsun-infected suntailed monkeys overlaps with that of mandrills in Gabon, south of the Ogoue River (Fig. 5). However, SIVsun is more distantly related to SIVmnd than to SIVlho and might not have been the proximal source of SIVmnd. Different hypotheses tried to explain this close relationship, and one of these involved a yet-unidentified SIV in another primate species (4). Given the relationship between SIVwrc and SIVlho/sun, another red colobus species could be involved. In Cameroon, the habitats from *Piliocolobus penantii preussi* overlap with that of mandrills and could be a possible candidate for the missing links. However, this area is also inhabited by the *Cercopithecus preussi* from the *lhoesti* superspecies, and the characterization of SIVs in this species will also provide more insights on the origin of SIVs in mandrills (Fig. 5). Overall, knowledge of primate behavior and past and recent geographic distribution of the different primate species could add important complementary information to understanding the evolutionary history of SIVs in nonhuman primates.

Importantly, humans and chimpanzees (*Pan troglodytes verus*) commonly hunt western red colobus for food (47), and humans also hunt chimpanzees. Numerous *P. troglodytes versus* samples have been analyzed, but no evidence for SIV infections has been reported yet, despite the high SIVwrc prevalence in their preys. The majority of these chimpanzee samples were obtained from wild caught animals, which are usually captured when infants (45, 51), in which the prevalences are generally lower. However, the absence of SIV infection could also be due to unadapted serologic and/or molecular tools, the majority of *P. troglodytes versus* samples have been screened with HIV-1-specific Western blots, which are maybe not able to efficiently detect cross-reactive antibodies of SIVwrc. The increasing acquisition of SIV sequences will allow us to develop new serologic and molecular tools in order to document with higher accuracy new SIV infections in wild Old word primates and to screen human populations to define whether cross-species transmissions with other SIVs occurred. The human population around the Taï forest still frequently hunts primates, and western red colobus represents an important part of the bushmeat monkeys (27, 42). Moreover, we previously documented high SIV prevalences in *P. badius badius* from the Taï forest (32). The ancestors of the two epidemic strains from HIV-2, group A and B, are derived from SIV that still circulate in wild mangabey populations from the Taï forest (44), illustrating the need for surveillance of primate pathogens and their cross-species transmissions in this part of Africa and elsewhere.

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